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Date: 24 Aug 01

By: Vladimir Skliba

**TRANSMITTAL LETTER TO THE UNITED STATES  
ELECTED OFFICE**

Applicant: Vincent J. WACHER, et al.

International Application No. PCT/US00/05524

Filing Date: March 1, 2000

Title of Invention: USE OF GALLIC ACID ESTERS TO INCREASE  
BIOAVAILABILITY OF ORALLY  
ADMINISTERED PHARMACEUTICAL  
COMPOUNDS

Priority Claimed March 5, 1999

1. Applicant herewith submits the following items under 35 USC 371 an express request to immediately begin national examination procedures (35 USC 371(f)).
2. Copy of the International application (35 USC 371(c)(2)):
  - ☐ is transmitted herewith.
  - ☒ is not required as the application was filed with the United States Receiving Office.
  - ☐ has been transmitted by the International Bureau. Date of mailing of the application (from form PCT/IB/308) is .
  - ☐ has been transmitted by applicant on .
3. Translation of the international application into the English language (35 USC 371(c)(2)):
  - ☒ is not required as the application was filed in English.
  - ☐ is transmitted herewith.
  - ☐ was previously transmitted by applicant on .

4. Amendments to the claims of the international application under PCT Article 19 (35 USC 371(c)(3)):

- ☐ are transmitted herewith.
- ☐ have been transmitted
  - ☐ by the International Bureau. Date on: .
  - ☐ by applicant on .
- ☒ have not been transmitted as
  - ☐ no notification has been received that the International Search Authority has received the Search Copy.
  - ☐ the Search Copy was received by the International Searching Authority but the Search Report has not yet been issued. Date of Receipt of Search Copy (from form PCT/ISA/202):
  - ☒ applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210): July 12, 2000.
  - ☐ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

5. Translation of the amendments to the claims under PCT Article 19 (35 USC 371(c)(3)):

- ☐ is transmitted herewith.
- ☐ is not required as the amendments were made in the English language.
- ☒ has not been transmitted for reasons indicated at paragraph 4 above.

6. Executed oath or declaration of the inventor (35 USC 371(c)(4)) complying with 35 USC 115:

- ☐ was previously submitted by applicant on .
- ☒ is submitted herewith and such oath or declaration
  - ☐ is attached to the application.
  - ☒ identifies the application and any amendments under PCT Article 19 which were transmitted as stated above; and states that they were reviewed by the inventor as required by 37 CFR 1.70.

7. International Search Report or Declaration under PCT Article 17(2)(a):

- ☐ is transmitted herewith.
- ☒ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): September 8, 2000.
- ☐ is not required as the application was searched by the United States International Searching Authority.
- ☐ will be transmitted promptly upon request.
- ☐ has been submitted by applicant on .

☐ is not transmitted as the international search has not yet issued.

8. Information Disclosure Statement under 37 CFR 1.97 and 1.98:

☐ was previously submitted by applicant on .

☐ is transmitted herewith.

☒ will be transmitted within three months of the date of submission of requirements under 35 USC 371(c).

9. A Verified Statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27:

☐ is transmitted herewith.

☐ was previously submitted by applicant on .

10. The above checked items are being transmitted:

☐ before the publication of the international application.

☐ after publication and the Article 20 communication but before 20 months from the priority date.

☐ after 20 months but before 22 months (surcharge and/or processing fee included).

☐ after 22 months (surcharge and/or processing fee included).

☒ by 30 months and a proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

☐ after 30 months but before 32 months (surcharge and/or processing fee included) and International Preliminary Examination was properly requested.

☐ after 32 months (surcharge and/or processing fee included).

11. Certain requirements under 35 USC 371 were previously submitted by the applicant on namely:

12. Other document(s) or information included:

☒ Power of Attorney by Assignee with copy of Assignment.

☒ Preliminary Amendment

13. Fee calculation:

☒ Basic National Fee (37 CFR 1.492(a)(1)-(4)):

☐ International preliminary examination fee paid to USPTO (37 CFR 1.492(a)(1)) (\$345 or \$690).

- ☐ No international preliminary examination fee paid to USPTO but international search fee paid to USPTO (37 CFR 1.492(a)(2)) (\$355 or \$710).
- ☐ Neither international preliminary examination fee nor international search fee paid to USPTO (37 CFR 1.492(a)(3)) (\$500 or \$1000).
- ☐ International preliminary examination fee paid to USPTO (37 CFR 1.492(a)(4)) and all claims satisfied provisions of PCT Article 33(1) to (4) (\$50 or \$100).
- ☒ Neither international preliminary examination fee nor international search paid to USPTO, but the Search Report was prepared by EPO (37 CFR 1.492(a)(5)) (\$430 or \$860).

**Total Basic National Stage Fee****\$860.00**

- ☒ Additional U.S. National Fee (35 USC 371(c)(1)) as follows:

FOR:	Claims Filed			Extra Claims <sup>1</sup>		Small Entity Rate Fee		Other Than a Small Entity Rate Fee		Total Filing Fee
Total Claims	30	-20=	10	\$9		\$18		\$180.00		
Independent Claims	3	-3=	0	\$40		\$80		\$0.00		
Multiple Dependent Claims Presented				\$135		\$270		\$0.00		
TOTAL										\$180.00

<sup>1</sup> If difference is negative, enter "0".

- ☒ Other fees as follows:
- ☐ Surcharge for furnishing the oath or declaration later than 20 months from any claimed priority date or later than 30 months and International Preliminary Examination was properly requested (37 CFR 1.492(e)).
- ☐ \$65.00 for a small entity
- ☐ \$130.00 for other than a small entity
- ☐ Processing fee of \$130.00 for furnishing the English Translation later than 20/30 months from any claimed priority date (37 CFR 1.492(f)).

**TOTAL FEES****\$1,040.00**

14. ☒ Payment of Fees

☒ [X] A check in the amount of \$1,040 to cover the above fees is enclosed.

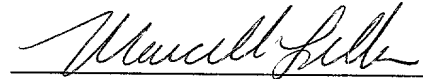
☐ [ ] Fees will be paid at a later time.

☐ [ ] The Commissioner is hereby authorized to charge underpayment of required fees under 37 CFR 1.16 or 37 CFR 1.17 associated with this communication or to credit any overpayment to Deposit Account No. 03-3117.

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Respectfully submitted,  
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By:

  
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09/914364

JC03 Rec'd PCT/PTO

24 AUG 2001  
PATENT

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Date:

24 Aug 01

By:



Vladimir Skliba

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Vincent J. Wachter Ph.D., et al.

Serial No.: Not yet assigned

Examiner:

Filed: Herewith

Art Unit:

International Application No.: PCT/US00/05524

Filed: March 1, 2000

For: USE OF GALLIC ACID ESTERS TO INCREASE BIOAVAILABILITY OF ORALLY ADMINISTERED PHARMACEUTICAL COMPOUNDS

Assistant Commissioner for Patents  
Washington, D.C. 20231

### PRELIMINARY AMENDMENT

Prior to examination of the above-identified application, the Examiner is respectfully requested to enter the following amendments.

#### IN THE CLAIMS

Please cancel claims 2-9, 13, 24-31, 37, 44-51, and 56.

Please replace claims 1, 23 and 43 with substitute claims 1, 23 and 43 below. A version of the claims with marking to show the changes made is attached.

1. (Amended) A method for increasing bioavailability of an orally administered pharmaceutical compound, the method comprising:

orally coadministering (1) the pharmaceutical compound to a mammal in need of treatment with the compound and (2) a gallic acid ester in an amount of the gallic acid ester sufficient to provide bioavailability of the compound in the presence of the gallic acid ester greater than bioavailability of the compound in the absence of the gallic acid ester, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid.

23. (Amended) A method of formulating an oral pharmaceutical composition, the method comprising:

admixing a pharmaceutical compound, a pharmaceutical carrier, and a gallic acid ester, the gallic acid ester being present in sufficient amount to provide bioavailability of the pharmaceutical compound in the presence of the gallic acid ester greater than the bioavailability of the pharmaceutical compound in the absence of the gallic acid ester when the pharmaceutical composition is administered orally to a mammal, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid.

43. (Amended) A method of increasing bioavailability of the active compound of an existing oral pharmaceutical composition, the method comprising:

reformulating the existing composition to provide a reformulated oral composition by admixing the active compound with a gallic acid ester, the gallic acid ester being present in sufficient amount to provide bioavailability of the active compound when administered in the reformulated composition greater than said bioavailability of the active compound when administered in the existing pharmaceutical composition, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid.

REMARKS

Independent claims 1, 23 and 43 have been amended to limit the gallic acid esters to those esters that are specifically recited in claims 9, 31 and 51 and claims 9, 31 and 51 have been cancelled. In addition, dependent claims reciting gallic acid esters other than the specifically recited esters above have been cancelled. No new matter is introduced by these amendments and their entry is respectfully requested.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 843-5000.

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Respectfully submitted,  
COOLEY GODWARD LLP

By:



\_\_\_\_\_  
Marcella Lillis, Ph.D.  
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VERSION OF CLAIMS WITH MARKINGS TO SHOW CHANGES MADE

1. (Amended) A method for increasing bioavailability of an orally administered pharmaceutical compound, the method comprising:
- orally coadministering (1) the pharmaceutical compound to a mammal in need of treatment with the compound and (2) a gallic acid ester in an amount of the gallic acid ester sufficient to provide bioavailability of the compound in the presence of the gallic acid ester greater than bioavailability of the compound in the absence of the gallic acid ester, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid [other than propyl gallate].
10. (Reiterated) The method of claim 1, wherein the gallic acid ester is coadministered in a range of 0.01 to 100 units of the gallic acid ester per 1 unit of the pharmaceutical compound.
11. (Reiterated) The method of claim 10, wherein the gallic acid ester is coadministered in a range of 0.1 to 10 units of the gallic acid ester per 1 unit of the pharmaceutical compound.
12. (Reiterated) The method of claim 11, wherein the gallic acid ester is coadministered in a range of 0.5 to 2 units of the gallic acid ester per 1 unit of the pharmaceutical compound.
14. (Reiterated) The method of claim 1, wherein the pharmaceutical compound is hydrophobic.

15. (Reiterated) The method of claim 1, wherein the amount is sufficient to produce a concentration of the gallic acid ester in the lumen of the gut of the mammal of at least 0.1 times a  $K_i$  or apparent  $K_i$  of CYP3A inhibition of the compound.

16. (Reiterated) The method of claim 1, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 10% of the difference between bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

17. (Reiterated) The method of claim 16, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 50% of the difference between bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

18. (Reiterated) The method of claim 17, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 75% of the difference between bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

19. (Reiterated) The method of claim 1, wherein the gallic acid ester shows an inhibition of at least 20% when the gallic acid ester and the compound are present in a 1:1 gallic acid ester:compound ratio.

20. (Reiterated) The method of claim 1, wherein the pharmaceutical compound comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazepine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide,

dibenzazepine, digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, pyridine, pyridone, pyrimidine, pyrrolidine, pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid, salicylate, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, or vinca alkaloid.

21. (Reiterated) The method of claim 1, wherein the gallic acid ester is present as a counter ion of the pharmaceutical compound.

22. (Reiterated) The method of claim 1, wherein the gallic acid ester is covalently bound to the pharmaceutical compound.

23. (Amended) A method of formulating an oral pharmaceutical composition, the method comprising:

admixing a pharmaceutical compound, a pharmaceutical carrier, and a gallic acid ester, the gallic acid ester being present in sufficient amount to provide bioavailability of the pharmaceutical compound in the presence of the gallic acid ester greater than the bioavailability of the pharmaceutical compound in the absence of the gallic acid ester when the pharmaceutical composition is administered orally to a mammal, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid [other than propyl gallate].

32. (Reiterated) The method of claim 23, wherein the gallic acid ester is present in a range of 0.01 to 100 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

33. (Reiterated) The method of claim 32, wherein the gallic acid ester is present in a range of 0.5 to 2 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

34. (Reiterated) The method of claim 23, wherein the gallic acid ester is present in an amount sufficient to produce a concentration of the gallic acid ester in the lumen of the gut of the mammal of at least 0.1 times a  $K_i$  or apparent  $K_i$  of CYP3A inhibition of the compound.

35. (Reiterated) The method of claim 23, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 10% of the difference between bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

36. (Reiterated) The method of claim 23, wherein the gallic acid ester is present in an amount sufficient to provide at least 1% by weight of the gallic acid ester relative to the total weight of the pharmaceutical composition.

38. (Reiterated) The method of claim 23, wherein the gallic acid ester is present as a counter ion of the pharmaceutical compound.

39. (Reiterated) The method of claim 23, wherein the gallic acid ester is covalently bound to the pharmaceutical compound.

40. (Reiterated) The method of claim 23, wherein the pharmaceutical compound comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazapine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide, dibenzazepine, digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, pyridine, pyridone, pyrimidine, pyrrolidine,

pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid, salicylate, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, or vinca alkaloid.

41. (Reiterated) A pharmaceutical composition produced by the process of claim 23.

42. (Reiterated) The composition of claim 41, wherein the gallic acid ester is present in an amount sufficient to provide at least 1% by weight of the gallic acid ester relative to the total weight of the pharmaceutical composition.

43. (Amended) A method of increasing bioavailability of the active compound of an existing oral pharmaceutical composition, the method comprising:  
reformulating the existing composition to provide a reformulated oral composition by admixing the active compound with a gallic acid ester, the gallic acid ester being present in sufficient amount to provide bioavailability of the active compound when administered in the reformulated composition greater than said bioavailability of the active compound when administered in the existing pharmaceutical composition, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid [other than propyl gallate].

52. (Reiterated) The method of claim 43, wherein the gallic acid ester is present in a range of 0.01 to 100 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

53. (Reiterated) The method of claim 52, wherein the gallic acid ester is present in a range of 0.5 to 2 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

54. (Reiterated) The method of claim 43, wherein the reformulated oral composition comprises all components present in the existing pharmaceutical composition plus the gallic acid ester.

55. (Reiterated) The method of claim 43, wherein the reformulated oral composition contains less than all components present in the existing pharmaceutical composition plus the gallic acid ester.

57. (Reiterated) A reformulated oral pharmaceutical composition produced by the process of claim 43.

## USE OF GALLIC ACID ESTERS TO INCREASE BIOAVAILABILITY OF ORALLY ADMINISTERED PHARMACEUTICAL COMPOUNDS

### INTRODUCTION

#### Technical Field

This invention is directed to the field of pharmacology and particularly to the formulation of oral pharmaceutical compositions for increased bioavailability and reduced inter- and intra-individual variability.

#### Background

Pharmacokinetics is the study of the fate of pharmaceuticals from the time they are ingested until they are eliminated from the body. The sequence of events for an oral composition includes absorption through the various mucosal surfaces, distribution via the blood stream to various tissues, biotransformation in the liver and other tissues, action at the target site, and elimination of drug or metabolites in urine or bile.

Bioavailability of a drug (pharmaceutical composition) following oral dosing is a critical pharmacokinetic determinant which can be approximated by the following formula:

$$F_{\text{oral}} = F_{\text{ABS}} \times F_{\text{G}} \times F_{\text{H}}$$

where  $F_{\text{oral}}$  is the oral bioavailability fraction, which is the fraction of the oral dose that reaches the circulation in an active, unchanged form.  $F_{\text{oral}}$  is less than 100% of the active ingredient in the oral dose for four reasons: (1) drug is not absorbed out of the gut lumen into the cells of the intestine and is eliminated in the feces; (2) drug is absorbed into the cells of the intestine but back-transported into the gut lumen; (3) drug is biotransformed by the cells of the intestine (to an inactive metabolite); or (4) drug is eliminated by the cells of the liver, either by biotransformation and/or by transport into the bile. Thus, oral bioavailability is the product of the fraction of the oral dose that is absorbed ( $F_{\text{ABS}}$ ), the fraction of the absorbed dose that successfully reaches the blood side of the gastrointestinal tract ( $F_{\text{G}}$ ), and the fraction of the drug in

the GI blood supply that reaches the heart side of the liver ( $F_H$ ). The extent of gut wall absorption, back transport and metabolism, and liver elimination are all subject to wide inter- and intra-individual variability.

Previous investigations arising in the laboratory of one of the present inventors resulted in new understandings of factors involved with bioavailability and in the invention described in U.S. Patent No. 5,567,592. The '592 patent describes general methods for increasing bioavailability of oral pharmaceutical compositions and methods for identifying compounds that increase bioavailability. However, although that invention made it possible to investigate a number of classes of compounds not previously thought to be useful in enhancing bioavailability, the actual process of identifying specific classes of compounds that are superior bioenhancers, among those bioenhancers which work to some degree, still remains a process of investigation and discovery. For example, the use of essential oils to enhance bioavailability of an orally administered pharmaceutical composition is disclosed in U.S. Patent No. 5,665,386.

### SUMMARY OF THE INVENTION

An object of this invention is to identify compositions with superior ability to increase drug bioavailability, particularly by increasing net drug absorption and/or decreasing drug biotransformation in the gut wall by inhibiting cytochrome P450 drug metabolism.

Another object of the invention is to provide compositions that strongly inhibit enzymes of the cytochrome P450 3A class (CYP3A) in the gut in preference to in other locations, such as the liver, which was previously thought to be the primary site of drug metabolism.

One specific object of the present invention is to reduce inter-individual variability of the systemic concentrations of the active pharmaceutical compound, as well as intra-individual variability of the systemic concentrations of the pharmaceutical compound being administered.

The invention is carried out by co-administering a gallic acid ester with an oral pharmaceutical compound (drug) or compounds to increase drug bioavailability. Particularly preferred esters are octyl gallate, lauryl gallate, and methyl gallate. The



compositions and methods of the invention can be used to increase drug efficacy in humans and in other mammals. Although veterinary use is specifically contemplated, the primary use will be in human treatment. Administration schemes include, but are not limited to, use of oral and topical formulations in humans and use of similar  
5 formulations for livestock.

### DESCRIPTION OF SPECIFIC EMBODIMENTS

#### Gallic Acid Esters Increase Drug Bioavailability

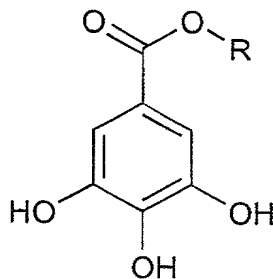
10 The present invention arises from continued research into the factors affecting drug bioavailability that were described in earlier applications arising from the laboratory of one of the present inventors. "Drug bioavailability" is defined here as the total amount of drug systemically available over time. The present inventors discovered previously that the single compound propyl gallate is useful in a method to  
15 increase the bioavailability of orally administered pharmaceutical compounds (U.S. Patent No. 5,962,522). Surprisingly, it has now been discovered that the class of compounds of gallic acid esters is useful in this regard. The present invention therefore provides a method for increasing drug bioavailability by inhibiting drug biotransformation in the gut using one or more of the compounds described herein.  
20 The compound(s) responsible for increased drug bioavailability is a gallic acid ester. The present inventors have discovered that gallic acid esters, in general, are capable of inhibiting the enzyme(s) responsible for drug biotransformation in the gut.

In general, the present invention provides a method for increasing the bioavailability of an orally administered pharmaceutical compound (particularly one  
25 which is hydrophobic) by orally co-administering the pharmaceutical compound to a mammal in need of treatment with an amount of a gallic acid ester sufficient to provide integrated systemic concentrations over time of the pharmaceutical compound greater than the integrated systemic concentrations over time of the pharmaceutical compound in the absence of the gallic acid ester. At least one gallic acid ester is  
30 utilized in the method of the invention to increase bioavailability. However, two or more gallic acid esters may be used simultaneously in the practice of the invention, leading to further increased bioavailability of the pharmaceutical compound, depending on the particulars of the compound. Changes in the integrated systemic

concentrations over time are indicated by "area under the curve" (AUC) measurements, an accepted pharmacological technique described in detail below.

#### Gallic acid esters

- 5 Gallic acid esters useful in the present invention have the general formula shown below:



(Formula I)

10

- The R group may be an alkyl (other than propyl), alkenyl, alkynyl, aryl, benzyl, phenyl, alicyclic, or heterocyclic group, all of which groups may be substituted or unsubstituted. R is preferably a C<sub>1</sub>-C<sub>22</sub> alkyl group (other than a propyl group), a C<sub>2</sub>-C<sub>22</sub> alkenyl group or a C<sub>2</sub>-C<sub>22</sub> alkynyl group, all of which groups may be substituted or unsubstituted and may be straight chain or branched chain. R is more preferably a C<sub>1</sub>-C<sub>12</sub> alkyl group, particularly a methyl, octyl or dodecyl (lauryl) group, or a C<sub>2</sub>-C<sub>18</sub> alkenyl group, particularly a cis-9-hexadecenyl (palmitoleyl), cis-9-octadecenyl (oleyl), cis,cis-9,12-octadecadienyl (linoleyl), trans,trans-9,12-octadecadienyl (linolelaidyl), cis,cis,cis-9,12,15-octadecatrienyl (linolenyl), trans,trans,trans-9,12,15-octadecatrienyl (linolenelaidyl), cis,cis,cis-6,9,12-octadecatrienyl (gamma-linolenyl), trans-9-octadecenyl (elaidyl) or trans-9-hexadecenyl (palmitelaidyl) group. Other gallic acid esters particularly useful in the present invention include (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid.

25

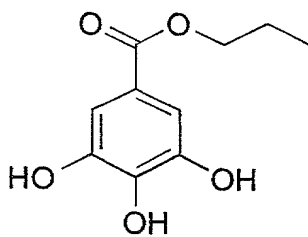
Many of the gallic acid esters used in the practice of the present invention are commercially available compounds or may be readily synthesized by methods that are well known in the art, for example, by refluxing gallic acid and the appropriate

alcohol (R-OH) in the presence of acid using standard conditions as described in Vogel, A., Vogel's Textbook of Organic Chemistry, 4<sup>th</sup> Edition, revised by Furniss, B.S. et al., Longman Inc., NY (1978). In a recently published example, lauryl gallate was prepared in greater than 90% yield by refluxing gallic acid and lauryl alcohol in dioxane in the presence of p-toluenesulfonic acid and zeolite (Chen, L. and Wu, K., *New process of synthesis of lauryl gallate*, Huaxue Shiji 19:382 (1997).

The gallic acid ester is preferably presented for coadministration in a ratio of gallic acid ester to drug in the range of 0.01 to 100 units gallic acid ester to 1 unit of the drug. For example, a formulation having 1 mg gallic acid ester per 100 mg drug represents the lower end of this range and a formulation having 500 mg gallic acid ester per 5 mg drug represents the upper end of this range. A more preferred range of gallic acid ester to drug in accordance with the present invention is 0.1 to 10 units gallic acid ester to 1 unit of the drug. The most preferred range is 0.5 to 2 units gallic acid ester 1 unit of the drug.

#### Properties of Gallic Acid Esters

The structure of propyl gallate (3,4,5-trihydroxybenzoic acid, n-propyl ester) is shown below:



(Formula II)

Propyl gallate has been used as an antioxidant or preservative in foods, drugs, cosmetics and pesticide products since 1948. This compound is Generally Recognized As Safe (GRAS) by the FDA and is listed in the Everything Added to Food in the United States (EAFUS) database as well as the United States Pharmacopeia-National Formulary (USP-NF) and the Food Chemicals Codex. The Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives has established an acceptable daily intake of 0-1.4 mg/kg/day for this compound. This value is 1/100 of the "no observed effect" level

determined in a 90 day feeding study in rats ("Gallates: Propyl, Octyl, Dodecyl," WHO Food Additive Series, 32:3-23 (1993)).

Octyl gallate and lauryl gallate have also been used as antioxidants in food; however, their current use is limited. The Joint Food and Agriculture

5 Organization/World Health Organization Expert Committee on Food Additives has established temporary acceptable daily intake levels of 0-0.1 mg/kg body weight for octyl gallate and 0-0.05 mg/kg body weight for lauryl gallate. These values are 1/200 of the "no observed effect level" determined in 90 day feeding studies in rats ("Gallates: Propyl, octyl, dodecyl." WHO Food Additive Series 32: 3-23 (1993);

10 Forty-first Report of the Joint FAO/WHO Expert Committee on Food Additives. *Evaluation of certain food additives and contaminants.* WHO Technical Report Series 837: 6, 46 (1993)). Lauryl gallate and octyl gallate are listed in the Everything Added to Food in the US (EAFUS) database, but neither of these compounds is listed in the United States Pharmacopeia-National Formulary (USP-NF) or the Food  
15 Chemical Codex.

Because these three alkyl gallate esters in very low concentrations, as have been used previously for the antioxidant purposes discussed above, are of low activity and thus not likely to be useful for the purposes described generally herein, only concentrations of a gallic acid ester providing an inhibition activity (resulting in  
20 increased bioavailability of a co-administered drug) are included in the invention. Preferred are those formulations of a gallic acid ester that show an inhibition of at least 20% at a 1:1 gallic acid ester:drug ratio; even more preferred are formulations of a gallic acid ester that show an inhibition of at least 50% at the same gallic acid ester to drug ratio.

#### 25 Bioavailability Measurements

The increase in drug bioavailability attributable to administration of the gallic acid ester can be determined by measuring total systemic drug concentrations over time after coadministration of a drug and the gallic acid ester and after administration  
30 of only the drug. The increase in drug bioavailability is defined as an increase in the Area Under the Curve (AUC). AUC is the integrated measure of systemic drug concentrations over time in units of mass-time/volume. The AUC from time zero (the time of dosing) to time infinity (when no drug remains in the body) following the

administration of a drug dose is a measure of the exposure of the patient to the drug. When efficacy of the gallic acid ester is being measured, the amount and form of active drug administered should be the same in both the coadministration of drug and gallic acid ester and the administration of the drug alone. For instance, administration of 10 mg of drug alone may result in total systemic drug delivered over time (as measured by AUC) of 500  $\mu\text{g}\cdot\text{hr}/\text{ml}$ . In coadministration (i.e., in the presence of the gallic acid ester) the systemic drug AUC may increase to 700  $\mu\text{g}\cdot\text{hr}/\text{ml}$ . If significantly increased drug bioavailability in the presence of the gallic acid ester is anticipated, drug doses may need to be reduced for safety.

Systemic drug concentrations are measured using standard drug measurement techniques. "Systemic drug concentration" refers to a drug concentration in a mammal's bodily fluids, such as serum, plasma or blood; the term also includes drug concentrations in tissues bathed by the systemic fluids, including the skin. Systemic drug concentration does not refer to digestive fluids. The increase in total systemic drug concentrations is one way of defining an increase of drug bioavailability due to coadministration of gallic acid ester and the drug. For drugs excreted in part unmetabolized in the urine, an increased amount of unchanged drug in the urine will reflect the increase in systemic concentrations.

#### Characteristics of Drugs Used with Gallic Acid Esters

The word "drug" as used herein is defined as a chemical capable of administration to an organism which modifies or alters the organism's physiology. More preferably the word "drug" as used herein is defined as any substance intended for use in the treatment or prevention of disease. Drug includes synthetic and naturally occurring toxins and bioaffecting substances as well as recognized pharmaceuticals, such as those listed in "The Physicians Desk Reference," 49th edition, 1995, pages 101-338; "Goodman and Gilman's The Pharmacological Basis of Therapeutics" 9th Edition (1996), pages 103-1645 and 1707-1792; and "The United States Pharmacopeia, The National Formulary," USP 23 NF 18 (1995), the compounds of these references being herein incorporated by reference. The term drug also includes compounds that have the indicated properties that are not yet discovered or available in the U.S. The term drug includes pro-active, activated and metabolized forms of drugs. The present invention can be used with drugs consisting of charged,

uncharged, hydrophilic, zwitter-ionic, or hydrophobic species, as well as any combination of these physical characteristics. A hydrophobic drug is defined as a drug which in its non-ionized form is more soluble in lipid or fat than in water. A preferred class of hydrophobic drugs is those drugs more soluble in octanol than in water.

In the method of the present invention, compounds (or drugs) from a number of classes of compounds that can be administered with gallic acid esters include, for example, the following classes: acetanilides, anilides, aminoquinolines, benzhydryl compounds, benzodiazepines, benzofurans, cannabinoids, cyclic peptides, dibenzazepines, digitalis glycosides, ergot alkaloids, flavonoids, imidazoles, quinolines, macrolides, naphthalenes, opiates (or morphinans), oxazines, oxazoles, phenylalkylamines, piperidines, polycyclic aromatic hydrocarbons, pyrrolidines, pyrrolidinones, stilbenes, sulfonylureas, sulfones, triazoles, tropanes, and vinca alkaloids.

#### Increased Drug Bioavailability by Inhibition of Cytochrome P450

##### **Phase I Biotransformation**

Inhibition of enterocyte cytochromes P450 participating in drug biotransformation is one objective of the present invention. The major enzymes involved in drug metabolism are present in the endoplasmic reticulum of many types of cells but are at the highest concentration in hepatocytes. Traditionally, enterocyte biotransformation was considered of minor importance in biotransformation compared to the liver. Many compounds inhibit cytochrome P450. These include, but are not limited to, ketoconazole, troleandomycin, gestodene, flavones such as quercetin and naringenin, erythromycin, ethynyl estradiol, and prednisolone. The primary goal of the invention is to use gallic acid ester to inhibit drug cytochrome P450 biotransformation in the gut to increase drug bioavailability.

##### **Types of Cytochromes and Tissue Location**

The cytochromes P450 are members of a superfamily of hemoproteins. They represent the terminal oxidases of the mixed function oxidase system. The cytochrome P450 gene superfamily is composed of at least 207 genes that have been named based on their evolutionary relationships. For this nomenclature system, the

sequences of all of the cytochrome P450 genes are compared, and those cytochromes P450 that share at least 40% identity are defined as a family (designated by *CYP* followed by a Roman or Arabic numeral, *e.g.* *CYP3*), further divided into subfamilies (designated by a capital letter, *e.g.* *CYP3A*), which are comprised of those forms that are at least 55% related by their deduced amino acid sequences (Nelson et al., *P450 superfamily: update on new sequences, gene mapping accession numbers and nomenclature*, Pharmacogenetics 6:1-42 (1996)). Finally, the gene for each individual form of cytochrome P450 is assigned an Arabic number (*e.g.* *CYP3A4*).

Three cytochrome P450 gene families (*CYP1*, *CYP2* and *CYP3*) appear to be responsible for most drug metabolism. At least 15 cytochromes P450 have been characterized to varying degrees in the human liver. At concentrations of the substrates found under physiologic conditions, enzyme kinetics often favor a single form of cytochrome P450 as the primary catalyst of the metabolism of a particular drug or other enzyme substrate.

The *CYP3* gene family encoding cytochromes P450 of type 3 is possibly the most important family in human drug metabolism. At least 5 forms of cytochrome P450 are found in the human 3A subfamily, and these forms are responsible for the metabolism of a large number of structurally diverse drugs. In non-induced individuals, 3A may constitute 20% of the P450 enzymes in the liver. In enterocytes, members of the 3A subfamily constitute greater than 70% of the cytochrome-containing enzymes. The present inventors have discovered that gallic acid esters preferentially inhibit the *CYP3A* form over the enzymes from the *CYP1* and *CYP2* families. The first two human 3A subfamily members identified were 3A3 and 3A4. These two cytochromes P450 are so closely related that the majority of studies performed to date have not been able to distinguish their contributions, and thus they are often referred to as 3A3/4. Erythromycin N-demethylation, cyclosporine oxidation, nifedipine oxidation, midazolam hydroxylation, testosterone 6 $\beta$ -hydroxylation, and cortisol 6 $\beta$ -hydroxylation are all *in vitro* probes of 3A3/4 catalytic activity. The levels of 3A3/4 vary by as much as 60-fold between human liver microsomal samples, with the levels of 3A forms approaching 50% of the total cytochrome P450 present in human liver samples from individuals receiving inducers of 3A3/4. The recently studied *CYP3A5* may also play a role as important as 3A3/4.

The liver contains many isoforms of cytochrome P450 and can biotransform a large variety of substances. The enterocytes lining the lumen of the intestine also have significant cytochrome P450 activity, and this activity is dominated by a single family of isozymes, 3A, the most important isoforms in drug metabolism.

5

### **Increased Drug Efficacy by Reducing CYP3A Drug Biotransformation**

The gallic acid ester, as used according to the invention, reduces drug biotransformation in the gut by inhibiting CYP3A activity in gut epithelial cells which leads to a total increase in drug bioavailability in the serum. In the presence of the gallic acid ester, fewer drug molecules will be metabolized by phase I enzymes in the gut and will not be available for phase II conjugation enzymes. This will lead to increased concentrations of untransformed drug passing from the gut into the blood and onto other tissues in the body.

Although the primary objective of the gallic acid ester is to inhibit CYP3A drug biotransformation in the gut, some biotransformation may be decreased in other tissues as well if the gallic acid ester is absorbed into the blood stream. The decrease in biotransformation by other tissues will also increase drug bioavailability. The advantage of targeting the gallic acid ester to the gut, however, is that it allows the use of lower systemic concentrations of the gallic acid ester compared to inhibitors that target CYP3A in the liver. After oral administration of the gallic acid ester, concentrations will be highest at the luminal surface of the gut epithelia, not having been diluted by systemic fluids and the tissues of the body. Luminal concentrations that are greater compared to blood concentrations will permit preferential inhibition of CYP3A in gut instead of the liver. Thus, as orally administered gallic acid esters preferentially inhibit CYP3A in the gut, they are a particularly effective means of increasing drug bioavailability of a co-administered drug.

Coadministration of the gallic acid ester will also reduce variability of oral bioavailability. Reduction of drug biotransformation or increased drug absorption will decrease variability of oral bioavailability to some degree because the increase in bioavailability will begin to approach the theoretical maximum of 100% oral bioavailability. The increase in oral bioavailability will be generally larger in subjects



with lower oral bioavailability. The result is a reduction in inter-individual and intra-individual variation. Addition of the gallic acid ester will reduce inter-individual and intra-individual variation of systemic concentrations of a drug or compound.

## 5    **A Net Increase in Drug Bioavailability Due to a Decrease in the Activity of CYP3A**

10    The catalytic activities of CYP3A that are subject to inhibition include, but are not limited to, dealkylase, oxidase, and hydrolase activities. In addition to the different catalytic activities of CYP3A, different forms of CYP3A exist with a range in molecular weight (for example, from 51 kD to 54 kD, as shown in Komori et al., J. Biochem., 104:912-16 (1988)).

15    The gallic acid esters reduce CYP3A drug biotransformation by acting as inhibitors of CYP3A activity. Possible mechanisms include competitive, non-competitive, uncompetitive, mixed or irreversible inhibition of CYP3A drug biotransformation.

## **Selection of Gallic Acid Ester Concentration by Reduction of CYP3A Drug Biotransformation**

20    The ability of the gallic acid ester to increase drug bioavailability of a particular drug can be estimated using *in vitro* and *in vivo* drug biotransformation measurements. *In vivo* measurements of drug bioavailability, such as measuring serum or blood drug concentrations over time, provide the closest measure of total drug systemic availability (bioavailability). *In vitro* assays of CYP3A metabolism indirectly indicate drug bioavailability because CYP3A drug metabolism affects  
25    integrated systemic drug concentrations over time. Although even a minimally measured increase is all that is required for a gallic acid ester to be useful, a preferred commercially desirable concentration of a gallic acid ester acting as a CYP3A modulator generally will increase drug bioavailability by at least 10%, preferably by at least 50%, and more preferably by at least 75% of the difference between  
30    bioavailability in its absence and complete oral bioavailability. For example, if the drug bioavailability is 40% without the gallic acid ester, then the addition of the gallic acid ester may increase bioavailability to 85%, for a 75% increase. A sufficient amount of orally administered gallic acid ester will provide integrated systemic drug

concentrations over time greater than the integrated systemic drug concentrations over time in the absence of the gallic acid ester. The actual amount or concentration of the gallic acid ester to be included with a pharmaceutical compound for a particular composition or formulation will vary with the active ingredient of the compound. The amount of the gallic acid ester to be used should be optimized using the AUC methods described herein, once the components for a particular pharmaceutical composition have been decided upon. As stated above, the recommended measure for the amount of the gallic acid ester in a particular formulation is by direct comparison to the amount of drug, with a gallic acid ester:drug ratio in the range of 0.01-100:1 being preferred, 0.1-10:1 being more preferred, and 0.5-2:1 being most preferred.

Inhibition of the P450 3A class of enzymes by gallic acid esters can be studied by a variety of bioassays, several of which are set forth below.

#### In vitro CYP3A Assays and Increased Drug Bioavailability

##### **Cell Assays of CYP3A Function and Increased Drug Bioavailability**

Cultured cells of either hepatocytes or enterocytes or freshly prepared cells from either liver or gut can be used to determine the activity of the gallic acid ester as a CYP3A inhibitor. Various methods of gut epithelial cell isolation can be used such as the method of Watkins et al., J. Clin. Invest., 80:1029-36 (1985). Cultured cells, as described in Schmiedlin-Ren et al., Biochem. Pharmacol., 46:905-918 (1993), can also be used. The production of CYP3A metabolites in cells can be measured using high pressure liquid chromatograph (HPLC) methods as described in the following section for microsome assays of CYP3A activity.

##### **Microsome Assays of CYP3A Function and Increased Bioavailability**

Microsomes from liver or intestine will be used for assays of CYP3A activity. Microsomes can be prepared from liver using conventional methods as discussed in Kronbach et al., Clin. Pharmacol. Ther., 43:630-5 (1988). Alternatively, microsomes can be prepared from isolated enterocytes using the method of Watkins et al., J. Clin. Invest., 80:1029-1037 (1987). Microsomes from gut epithelial cells can also be prepared using calcium precipitation as described in Bonkovsky et al., Gastroenterology, 88:458-467 (1985). Microsomes can be incubated with drugs and the metabolites monitored as a function of time. In addition, the levels of these

enzymes in tissue samples can be measured using radioimmunoassays or western blots. Additionally, the production of metabolites can be monitored using high pressure liquid chromatography systems (HPLC) and identified based on retention times. CYP3A activity can also be assayed colorimetrically measuring erythromycin demethylase activity as the production of formaldehyde as in Wrighton et al., Mol. Pharmacol., 28:312-321 (1985) and Nash, Biochem. J., 55:416-421 (1953).

### Characteristics of Gallic Acid Esters for Reducing CYP3A Drug Metabolism

Gallic acid esters bind CYP3A quickly and inhibit while the drug is passing through the enterocyte. After the gallic acid ester reaches the heart and is distributed throughout the body the concentration of the gallic acid ester will be diluted on future passes through the liver. Concentrations of gallic acid ester used in the gut lumen are preferably selected to be effective on gut CYP3A metabolism but, due to dilution, to be less active in other tissues.

The amount of the gallic acid ester used for oral administration can be selected to achieve small intestine luminal concentrations of at least 0.1 times the  $K_i$  or apparent  $K_i$  for CYP3A inhibition of drug metabolism or an amount sufficient to increase systemic drug concentration levels, whichever is less. Alternatively, the amount of a gallic acid ester inhibitor of cytochrome P450 3A enzyme that will be used in a formulation can be calculated by various assays that are described in detail below. For example, one such assay measures the conversion of nifedipine to its oxidation product in an assay system containing 50-500  $\mu$ g human liver microsomes, 10-100  $\mu$ M nifedipine, and 1mm NADPH in 500  $\mu$ l of 0.1 M sodium phosphate buffer, pH 7.4. In the practice of the method of the present invention, the initial amount of gallic acid ester is selected to provide concentrations in the lumen of the small intestine equal to or greater than concentrations that reduce the rate of conversion determined by this assay, preferably a rate reduction of at least 10%. While the actual dose of gallic acid ester in a clinical formulation might be optimized from this initial dosage depending on the results of a clinical trial, the assay as described is sufficient to establish a utilitarian dosage level.

In all of these cases, the goal in selecting a particular concentration of a gallic acid ester is increased bioavailability of the pharmaceutical compound that is being administered. Thus, a desirable goal is to provide integrated systemic concentrations

over time of the pharmaceutical compound in the presence of the gallic acid ester that is greater than the integrated systemic concentrations over time of the pharmaceutical compound in the absence of the gallic acid ester by at least 10% of the difference between bioavailability in its absence and complete oral bioavailability. Preferred is attainment of "complete bioavailability," which is 100% systemic bioavailability of the administered dosage.

#### Screening Assay for Superior Gallic Acid Ester Formulations

In summary, the various techniques described above for screening gallic acid ester concentrations for activity levels by assaying for inhibition in the gut of a mammal of activity of a cytochrome P450 enzyme are all generally useful as methods of creating useful formulations that are most useful for increasing bioavailability of the active ingredient of a given drug in a mammal. In all of these assays, the best amounts are those that best inhibit enzymatic destruction of a tested drug in the gut of the mammal (either by direct testing *in vivo* or by a test that predicts such activity). When testing for inhibition of activity of a cytochrome enzyme, assays that detect inhibition of members of a cytochrome P450 3A family (for a particular mammal, particularly human) are preferred. Although *in vivo* assays are preferred because of the direct relationship between the measurement and gut activity, other assays, such as assays for inhibition of cytochrome P450 activity in isolated enterocytes or hepatocytes or microsomes obtained from either enterocytes or hepatocytes of the mammal in question or for inhibition of cytochrome P450 in a tissue or membrane from the gut of said mammal, are still useful as screening assays. It is possible to use enzymes from both the gut and liver interchangeably for these assays since it has been shown that CYP3A enzymes are identical in the two locations (Kolars, J.C. et al., *Identification of Rifampin-Inducible P450III A4 (CYP3A4) in Human Small Bowel Enterocytes*, *J. Clin. Investig.*, 90:1871-1878 (1992); Lown, K.S. et al., *Sequences of intestinal and hepatic cytochrome p450 3A4 cDNAs are identical*, *Drug Metab. Dispos.*, 26:185-187(1998)).

#### Coadministration and Delivery of the Gallic Acid Ester

#### **Coadministration of the Gallic Acid Ester and a Drug**

The present invention will increase the bioavailability of a drug in systemic fluids or tissues by co-administering the gallic acid ester with a drug. "Co-administration" includes concurrent administration (administration of the gallic acid ester and drug at the same time) and time-varied administration (administration of the gallic acid ester at a time different from that of the drug), as long as both the gallic acid ester and the drug are present in the gut lumen and/or membranes during at least partially overlapping times. "Systemic fluids or tissues" refers to blood, plasma, or serum and to other body fluids or tissues in which drug measurements can be obtained.

### **Delivery Vehicles and Methods**

Coadministration can occur with the same delivery vehicle or with different delivery vehicles. The gallic acid ester and the drug can be administered using, as examples, but not limited to, time release matrices, time release coatings, companion ions, and successive oral administrations. Alternatively, the drug and the gallic acid ester can be separately formulated with different coatings possessing different time constants for release of the gallic acid ester and the drug. The gallic acid ester can also be bound to the drug being protected, either by covalent bonding or by ionic or polar attractions.

The gallic acid ester also increases bioavailability when used with epithelia tissues other than the gut. The discussion above of the invention as used in the gut is appropriate for other types of epithelia. For example, CYP 3A enzymes are present in the skin, and a gallic acid ester can be used in transdermal formulations to increase drug bioavailability to systemic fluids and tissues. Such applications are part of the invention, since inhibition of CYP 3A enzymes by a gallic acid ester in epithelia other than the gut provides the same mechanism of action.

### **Formulations Having a Gallic Acid Ester**

The invention is carried out in part by formulating an oral pharmaceutical composition to contain at least one gallic acid ester. This is accomplished in some embodiments by admixing a pharmaceutical compound, usually a pharmaceutical carrier, and the gallic acid ester, the gallic acid ester being present in an amount sufficient to provide integrated systemic concentrations over time of the

pharmaceutical compound (as measured by AUCs greater than the integrated systemic concentrations over time of the pharmaceutical compound in the absence of the gallic acid ester) when the pharmaceutical composition is administered orally to an animal being treated. Additionally, more than one gallic acid ester may be used in the formulation. A pharmaceutical carrier is generally an inert bulk agent added to make the active ingredients easier to handle and can be solid or liquid in the usual manner as is well understood in the art. Pharmaceutical compositions produced by the process described herein are also part of the present invention.

The present invention can also be used to increase the bioavailability of the active compound of an existing oral pharmaceutical composition. When practiced in this manner, the invention is carried out by reformulating the existing composition to provide a reformulated composition by admixing the active compound with the gallic acid ester, the gallic acid ester being present in an amount sufficient to provide integrated systemic concentrations over time of the compound when administered in the reformulated composition greater than the integrated systemic concentrations over time of the compound when administered in the existing pharmaceutical composition. All of the criteria described for new formulations also apply to reformulation of old compositions. In preferred aspects of reformulations, the reformulated composition comprises all components present in the existing pharmaceutical composition plus the gallic acid ester, thus simplifying practice of the invention, although it is also possible to eliminate existing components of formulations because of the increase in bioavailability. Thus, the invention also covers reformulated compositions that contain less than all components present in the existing pharmaceutical composition plus the gallic acid ester. However, this invention does not cover already existing compositions that contain a component which increases bioavailability by mechanisms described in this specification (without knowledge of the mechanisms), should such compositions exist.

Traditional formulations can be used with the gallic acid ester. Optimal gallic acid ester concentrations can be determined by varying the amount and timing of gallic acid ester administration and monitoring bioavailability. Once the optimal gallic acid ester concentration or gallic acid ester to drug ratio is established for a particular drug, the formulation (gallic acid ester, drug, and other formulation components, if any) is tested clinically to verify the increased bioavailability. In the

case of time- or sustained- release formulations, it will be preferred to establish the optimal gallic acid ester concentration using such formulations from the start of the bioavailability experiments.

Several gallic acid esters have been used as antioxidants under many different circumstances, including as part of a pharmaceutical composition or formulation.

Their use has been limited to preventing decomposition of the materials in the formulation, rather than for a physiological effect. As antioxidants, gallic acid esters are used in small quantities, and such materials are not likely to approach even the outer limits of the present invention as defined by the specification and claims. In particular, preferred formulations of the invention contain at least 1% by weight gallic acid ester relative to the total weight of the formulation (including the capsule, if present), more preferably at least 2%, even more preferably at least 5%. For example, propyl gallate, when used as an antioxidant, is used in an amount that is less than 0.1% of the materials being protected or preserved. Other gallic acid esters, for example octyl gallate or lauryl gallate, are used as antioxidants at equivalent or lower levels. In considering these percentages, it should be recalled that these are percentages of the formulation in which the active ingredient is being presented, not percentages by weight or volume as concentrations in the medium in which the pharmaceutical composition will become dissolved or suspended after ingestion of the formulation. Furthermore, the gallic acid ester may be used in capsules (either hard or soft standard pharmaceutical gel capsules, for example).

The invention now being generally described, the same will be better understood by reference to the following detailed example, which is offered for illustration only and is not to be considered limiting of the invention unless otherwise specified.

## EXAMPLES

### Example 1: Inhibition of Drug Degradation by Gallic Acid Esters

The known CYP3A substrate nifedipine (Gonzalez, F.J., et al., *Human P450PCN1: sequence, chromosome localization, and direct evidence through cDNA expression that P450PCN1 is nifedipine oxidase*, DNA, 2:79-86 (1988)) was used as a

test substrate for evaluating the potential of various gallic acid esters to inhibit CYP3A metabolism in a human liver microsome study.

To prepare the microsomes, human liver pieces were perfused with 1.15% potassium chloride then homogenized in 0.1 mM Tris-acetate, pH 7.4, containing 1mM EDTA and 20 mM BHT. Microsomal pellets were prepared from the homogenate using standard differential centrifugation procedures (Guengerich, *Analysis and characterization of enzymes in Principles and Methods of Toxicology*, A.W. Hayes (ed.), Raven Press, New York. pp. 777-814 (1989)) and were stored at -80°C in Tris-acetate buffer, pH 7.4, containing 20% w/v glycerol. Microsomes were diluted in 100 mM potassium phosphate buffer, pH 7.4, for use in metabolic incubations. Microsomal protein and CYP content of the human liver microsomes were determined using methods of Bradford (Bradford, M.M. *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding*. Anal. Biochem. 72:248-254 (1976)) and Omura and Sato (Omura, T. et. al. *The carbon monoxide-binding pigment of liver microsomes II. Solubilization, purification and properties*. J. Biol. Chem. 239:2370-2378 (1964)), respectively.

In the experiments, 100 µM nifedipine and either 5 µl of a solution of one of the gallic acid esters (at a concentration indicated in **Table 1**) or 5 µl of solvent alone (control) were preincubated along with 0.1 mg/ml of the human liver microsomal proteins and 1 mM diethylenetriaminepentaacetic acid (DETAPAC) in 100 mM phosphate buffer, pH 7.4, for 5 minutes at 37 °C. Metabolic reactions were started by addition of reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) to give a final concentration of 1 mM and a final volume of 0.5 ml. Metabolic reactions were stopped after 3 minutes by vortex mixing with 0.2 ml of an extraction solvent of (94:6) acetonitrile:glacial acetic acid. Protein was precipitated by centrifugation (3000 rpm x 10 minutes) and supernatants were analyzed for nifedipine and its oxidation product 2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester by High Performance Liquid Chromatography (HPLC).

All experiments were conducted in triplicate and compared to incubations carried out without NADPH or without substrate. The data are the mean ± standard deviation of three measurements.



The results, shown in Table 1, indicate that nifedipine oxidation rates in the presence of all gallic acid esters tested at the indicated concentrations were significantly different from the control ( $P < 0.05$ ) using ANOVA with Dunnett's post hoc comparison.

Table 1

Inhibition of CYP3A-Mediated Metabolism in  
Human Liver Microsomes by Gallic Acid Esters

Inhibitor <sup>a</sup>	Con c. ( $\mu$ M)	Relative Nifedipine Oxidation Rate ( $\pm$ S.D.)	Comment
Control	--	100 (3)	
Methyl gallate	100 500	53 (2) 16.0 (0.1)	
Propyl gallate	50 100 500	59 (2) 34 (1) 6.1 (0.1)	Non-competitive inhibitor of nifedipine metabolism $K_i = 64 \pm 2 \mu\text{M}$ (mean $\pm$ S.E. of estimate; $r^2 = 0.999$ ) <sup>b</sup>
Octyl gallate	10 25 50 100	44 (2) 17.5 (0.5) 4.9 (0.6) 1.1 (0.3)	Non-competitive inhibitor of nifedipine metabolism $K_i = 5.2 \pm 0.2 \mu\text{M}$ (mean $\pm$ S.E. of estimate; $r^2 = 0.996$ ) <sup>b</sup>
Lauryl gallate (Dodecyl gallate)	10 25 50 100	61 (1) 31 (3) 12.6 (0.1) 6.4 (0.5)	

<sup>a</sup> Substrate and inhibitor were dissolved in acetonitrile except for lauryl gallate which required methanol as vehicle.

<sup>b</sup> Inhibition constant  $K_i$  determinations utilized 10, 20, 50 and 100  $\mu\text{M}$  nifedipine substrate concentrations and experiments were run in duplicate.  $K_i$  values were determined by regression analysis of rate data using SigmaPlot V4.OS software (SPSS Inc., San Rafael, California).

The various gallic acid esters, at all tested concentrations, served as effective inhibitors of CYP3A-mediated metabolism. Octyl gallate proved to be an especially good inhibitor of the metabolism at relatively low concentrations. The usefulness of gallic acid esters to increase bioavailability of pharmaceutical compounds given to patients by coadministration of the gallic acid ester with the pharmaceutical compound is thus self-evident.

### Example 2: Inhibition of Drug Degradation by Propyl Gallate

The ability of propyl gallate at various concentrations to inhibit metabolism for three representative drugs through inhibition of the cytochrome P450 mechanism was tested. Human liver microsomes were prepared and each of three drugs, amiodarone, buspirone, or nifedipine, were incubated with the microsomes in the presence of propyl gallate or a known inhibitor of CYP3A metabolism. Metabolism in the presence of propyl gallate or known CYP3A inhibitor was compared to a control treated only with the solvent in which the inhibitor was dissolved.

Inhibition of metabolism of the known CYP3A substrates amiodarone (Fabre, G., et al., *Evidence for CYP3A-mediated N-deethylation of amiodarone in human liver microsomal fractions*, Drug Metab. Dispos., 21:978-985 (1993), Triver, J.M., et al., *Amiodarone N-deethylation in human liver microsomes: involvement of cytochrome P450 3A enzymes (first report)*, Life Sci., 52:PL91-96 (1993)), nifedipine (Gonzalez, F.J., et al., *Human P450PCN1: sequence, chromosome localization, and direct evidence through cDNA expression that P450PCN1 is nifedipine oxidase*, DNA, 2:79-86 (1988)), and buspirone (Kivistö K.T. et al. *Plasma buspirone concentrations are greatly increased by erythromycin and itraconazole*. Clin. Pharmacol. Ther. 62: 348-54 (1997); Lilja J.J. *Grapefruit juice substantially increases plasma concentrations of buspirone*. Clin. Pharmacol. Ther. 64: 655-60 (1998)) by human liver microsomes was tested. The microsomes were prepared as in Example 1.

The amiodarone was present in a concentration of 100  $\mu$ M, the buspirone was in a concentration of 25  $\mu$ M, and the nifedipine was present in a concentration of 25  $\mu$ M. The propyl gallate was tested with each of these drugs at concentrations of 25, 50, and 100  $\mu$ M. Other inhibitors of CYP3A metabolism were utilized at known inhibition concentrations, i.e. ketoconazole at 1  $\mu$ M, cyclosporine at 25  $\mu$ M, and diltiazem, erythromycin, and verapamil at 100  $\mu$ M.

The drug and optionally the inhibitor were preincubated with the microsomes at 1 nmol CYP/ml and 1 mM DETAPAC in 100 mM phosphate buffer, pH 7.4 for 5 minutes at 37°C. After the preincubation, metabolic reactions were started by the addition of 1 mM NADPH. Samples were taken at 1, 2, and 3 minutes after the start of the reaction and analyzed by HPLC. Disappearance of substrate and/or formation of metabolite were quantitated by comparison to standard curves.

The results are presented in **Table 2**. The metabolism rates (nmol/ml/min) are the mean  $\pm$  standard deviation of three measurements. Also shown in **Table 2** are the metabolism rates expressed as a percentage of the control for each drug. These numbers are presented in parentheses.

5

**Table 2**

Inhibition of CYP3A-Mediated Metabolism in  
Human Liver Microsomes by Propyl Gallate

Inhibitor	$\mu$ M	Mean $\pm$ SD Metabolism rate (% control)		
		Amiodarone <sup>a</sup>	Buspirone <sup>b</sup>	Nifedipine <sup>c</sup>
Control		1.92 $\pm$ 0.08 (100)	5.37 $\pm$ 0.56 (100)	4.36 $\pm$ 0.17 (100)
Propyl Gallate	25	0.94 $\pm$ 0.02 (49)	3.49 $\pm$ 0.49 (65)	3.57 $\pm$ 0.29 (82)
	50	0.55 $\pm$ 0.02 (28)	2.25 $\pm$ 0.25 (42)	2.35 $\pm$ 0.10 (54)
	100	0.32 $\pm$ 0.03 (17)	1.55 $\pm$ 0.23 (29)	1.43 $\pm$ 0.04 (33)
Ketoconazole	1	0.79 $\pm$ 0.004 (4)	1.47 $\pm$ 0.39 (28)	0.48 $\pm$ 0.06 (11)
Cyclosporine	25	0.32 $\pm$ 0.03 (17)	2.21 $\pm$ 0.38 (41)	1.05 $\pm$ 0.02 (24)
Diltiazem	100	1.06 $\pm$ 0.02 (55)	2.80 $\pm$ 0.18 (52)	3.74 $\pm$ 0.16 (86)
Erythromycin	100	0.84 $\pm$ 0.07 (44)	3.59 $\pm$ 0.46 (67)	2.67 $\pm$ 0.11 (61)
Verapamil	100	0.81 $\pm$ 0.04 (42)	2.34 $\pm$ 0.46 (44)	3.00 $\pm$ 0.05 (69)

<sup>a</sup> Formation rate of N-desethylamiodarone metabolite (nmol/ml/min)

<sup>b</sup> Buspirone disappearance (nmol/ml/min)

<sup>c</sup> Formation of nifedipine oxidation product 2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylic acid, dimethyl ester (nmol/ml/min)

As evidenced above, propyl gallate at all tested concentrations and against each drug, served as an effective inhibitor of CYP3A-mediated metabolism. Greater inhibition of the metabolism occurred with increasing concentrations of propyl gallate. Propyl gallate also compared favorably with the known CYP3A inhibitors tested. Specifically, propyl gallate was found to be better at inhibiting drug metabolism than the established CYP3A inhibitors diltiazem, erythromycin, and

verapamil. This demonstrates the utility of propyl gallate to increase bioavailability of compounds by coadministration of propyl gallate with a pharmaceutical compound.

### Example 3: Inhibition of Nifedipine Degradation by Heterocyclic Gallic Acid Esters

Four commercially available gallic acid esters were for inhibition of CYP3A as shown by their effect on nifedipine oxidation by human liver microsomes as described in Example 1. The results of these assays are shown in the Table 3 below.

TABLE 3

Effect of gallic acid esters with heterocyclic R groups on nifedipine oxidation by human liver microsomes.

<i>Inhibitor</i>	<i>Relative nifedipine oxidation (<math>\pm</math>S.D.) at indicated inhibitor Concentration (n=3)*</i>				
	0 (control)	10 $\mu$ m	25 $\mu$ m	50 $\mu$ m	100 $\mu$ m
(-)-Epicatechin gallate	100(4)	73(1)	58(1)	40(1)	30.7(0.2)
(-)-Epigallocatechin gallate	100(4)	83.0(0.4)	62(4)	44(2)	25(2)
(-)-Gallocatechin gallate	100(4)	64(1)	37(1)	15(1)	9(1)
Tannic acid (also called tannin)	100(4)	63(2)	37(3)	13(1)	0(0)

- A smaller number indicates greater inhibition of metabolism. Metabolism in the presence of inhibitor at all concentrations shown was statistically significant as compared to the control ( $P>0.05$ ) as determined by ANOVA with Dunnett's post-hoc comparison.

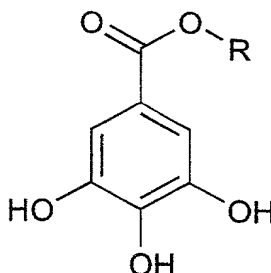
All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WE CLAIM:

1. A method for increasing bioavailability of an orally administered pharmaceutical compound, the method comprising:

5 orally coadministering (1) the pharmaceutical compound to a mammal in need of treatment with the compound and (2) a gallic acid ester in an amount of the gallic acid ester sufficient to provide bioavailability of the compound in the presence of the gallic acid ester greater than bioavailability of the compound in the absence of the gallic acid ester, wherein said gallic acid ester is other than  
10 propyl gallate.

2. The method of claim 1, wherein the gallic acid ester has the formula



15 wherein R is a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, benzyl, phenyl, alicyclic or heterocyclic group.

3. The method of claim 2, wherein R is a substituted or unsubstituted alkyl, alkenyl or alkynyl group.

20 4. The method of claim 3, wherein R is a C<sub>1</sub>-C<sub>22</sub> alkyl group or a C<sub>2</sub>-C<sub>22</sub> alkenyl group.

5. The method of claim 4, wherein R is a C<sub>1</sub>-C<sub>12</sub> alkyl group.

25 6. The method of claim 5, wherein R is selected from the group consisting of a methyl group, an octyl group, and a lauryl group.

7. The method of claim 4, wherein R is a C<sub>2</sub>-C<sub>18</sub> alkenyl group.

8. The method of claim 4, wherein R is selected from the group consisting of a methyl group, an octyl group, a lauryl group, a cis-9-hexadecenyl group, a cis-9-octadecenyl group, a cis,cis-9,12-octadecadienyl group, a trans,trans-9,12-octadecadienyl group, a cis,cis,cis-9,12,15-octadecatrienyl group, a trans,trans,trans-9,12,15-octadecatrienyl group, a cis,cis,cis-6,9,12-octadecatrienyl group, a trans-9-octadecenyl group, and a trans-9-hexadecenyl group.

9. The method of claim 1, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid.

10. The method of claim 1, wherein the gallic acid ester is coadministered in a range of 0.01 to 100 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

11. The method of claim 10, wherein the gallic acid ester is coadministered in a range of 0.1 to 10 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

12. The method of claim 11, wherein the gallic acid ester is coadministered in a range of 0.5 to 2 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

13. The method of claim 1, wherein the gallic acid ester comprises at least two of octyl gallate, lauryl gallate, and methyl gallate.

14. The method of claim 1, wherein the pharmaceutical compound is hydrophobic.

15. The method of claim 1, wherein the amount is sufficient to produce a concentration of the gallic acid ester in the lumen of the gut of the mammal of at least 0.1 times a  $K_i$  or apparent  $K_i$  of CYP3A inhibition of the compound.

5 16. The method of claim 1, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 10% of the difference between bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

10 17. The method of claim 16, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 50% of the difference between bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

15 18. The method of claim 17, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 75% of the difference between bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

20 19. The method of claim 1, wherein the gallic acid ester shows an inhibition of at least 20% when the gallic acid ester and the compound are present in a 1:1 gallic acid ester:compound ratio.

25 20. The method of claim 1, wherein the pharmaceutical compound comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazapine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide, dibenzazepine, digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, 30 oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, pyridine, pyridone, pyrimidine, pyrrolidine,



pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid, salicylate, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, or vinca alkaloid.

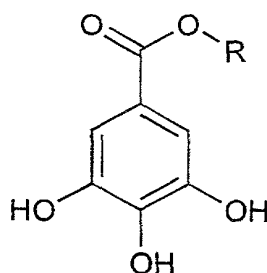
21. The method of claim 1, wherein the gallic acid ester is present as a counter ion of the pharmaceutical compound.

22. The method of claim 1, wherein the gallic acid ester is covalently bound to the pharmaceutical compound.

23. A method of formulating an oral pharmaceutical composition, the method comprising:

admixing a pharmaceutical compound, a pharmaceutical carrier, and a gallic acid ester, the gallic acid ester being present in sufficient amount to provide bioavailability of the pharmaceutical compound in the presence of the gallic acid ester greater than the bioavailability of the pharmaceutical compound in the absence of the gallic acid ester when the pharmaceutical composition is administered orally to a mammal, wherein said gallic acid ester is other than propyl gallate.

24. The method of claim 23, wherein the gallic acid ester has the formula



wherein R is a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, benzyl, phenyl, alicyclic or heterocyclic group.

25. The method of claim 24, wherein R is a substituted or unsubstituted alkyl, alkenyl or alkynyl group.

26. The method of claim 25, wherein R is a C<sub>1</sub>-C<sub>22</sub> alkyl group or a C<sub>2</sub>-C<sub>22</sub> alkenyl group.

27. The method of claim 26, wherein R is a C<sub>1</sub>-C<sub>12</sub> alkyl group.

28. The method of claim 27, wherein R is selected from the group consisting of a methyl group, an octyl group, and a lauryl group.

29. The method of claim 26, wherein R is a C<sub>2</sub>-C<sub>18</sub> alkenyl group.

30. The method of claim 26, wherein R is selected from the group consisting of a methyl group, an octyl group, a lauryl group, a cis-9-hexadecenyl group, a cis-9-octadecenyl group, a cis,cis-9,12-octadecadienyl group, a trans,trans-9,12-octadecadienyl group, a cis,cis,cis-9,12,15-octadecatrienyl group, a trans,trans,trans-9,12,15-octadecatrienyl group, a cis,cis,cis-6,9,12-octadecatrienyl group, a trans-9-octadecenyl group, and a trans-9-hexadecenyl group.

31. The method of claim 23, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid.

32. The method of claim 23, wherein the gallic acid ester is present in a range of 0.01 to 100 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

33. The method of claim 32, wherein the gallic acid ester is present in a range of 0.5 to 2 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

34. The method of claim 23, wherein the gallic acid ester is present in an amount sufficient to produce a concentration of the gallic acid ester in the lumen of the gut of the mammal of at least 0.1 times a K<sub>i</sub> or apparent K<sub>i</sub> of CYP3A inhibition of the compound.

35. The method of claim 23, wherein bioavailability of the compound in the presence of the gallic acid ester is greater than bioavailability of the compound in the absence of the gallic acid ester by at least 10% of the difference between  
5 bioavailability in the absence of the gallic acid ester and complete oral bioavailability.

36. The method of claim 23, wherein the gallic acid ester is present in an amount sufficient to provide at least 1% by weight of the gallic acid ester relative to  
10 the total weight of the pharmaceutical composition.

37. The method of claim 23, wherein the gallic acid ester comprises at least two of octyl gallate, lauryl gallate, and methyl gallate.

38. The method of claim 23, wherein the gallic acid ester is present as a counter ion of the pharmaceutical compound.

39. The method of claim 23, wherein the gallic acid ester is covalently bound to the pharmaceutical compound.

40. The method of claim 23, wherein the pharmaceutical compound comprises an acetanilide, aminoacridine, aminoquinoline, anilide, anthracycline antibiotic, antiestrogen, benzazepine, benzhydryl compound, benzodiazepine, benzofuran, cannabinoid, cephalosporine, colchicine, cyclic peptide, dibenzazepine,  
25 digitalis glycoside, dihydropyridine, epiphodophyllotoxin, ergeline, ergot alkaloid, imidazole, isoquinoline, macrolide, naphthalene, nitrogen mustard, opioid, oxazine, oxazole, phenothiazine, phenylalkylamine, phenylpiperidine, piperazine, piperidine, polycyclic aromatic hydrocarbon, pyridine, pyridone, pyrimidine, pyrrolidine, pyrrolidinone, quinazoline, quinoline, quinone, rauwolfia alkaloid, retinoid,  
30 salicylate, steroid, stilbene, sulfone, sulfonylurea, taxol, triazole, tropane, or vinca alkaloid.

41. A pharmaceutical composition produced by the process of claim 23.

42. The composition of claim 41, wherein the gallic acid ester is present in an amount sufficient to provide at least 1% by weight of the gallic acid ester relative to the total weight of the pharmaceutical composition.

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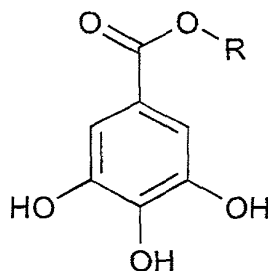
43. A method of increasing bioavailability of the active compound of an existing oral pharmaceutical composition, the method comprising:

reformulating the existing composition to provide a reformulated oral composition by admixing the active compound with a gallic acid ester, the gallic acid ester being present in sufficient amount to provide bioavailability of the active compound when administered in the reformulated composition greater than said bioavailability of the active compound when administered in the existing pharmaceutical composition, wherein said gallic acid ester is other than propyl gallate.

10

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44. The method of claim 43, wherein the gallic acid ester has the formula



wherein R is a substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, phenyl, benzyl, alicyclic or heterocyclic group.

20

45. The method of claim 44, wherein R is a substituted or unsubstituted alkyl, alkenyl or alkynyl group.

25

46. The method of claim 45, wherein R is a C<sub>1</sub>-C<sub>22</sub> alkyl group or a C<sub>2</sub>-C<sub>22</sub> alkenyl group.

47. The method of claim 46, wherein R is a C<sub>1</sub>-C<sub>12</sub> alkyl group.

48. The method of claim 47, wherein R is selected from the group consisting of a methyl group, an octyl group, and a lauryl group.

49. The method of claim 46, wherein R is a C<sub>2</sub>-C<sub>18</sub> alkenyl group.

50. The method of claim 46, wherein R is selected from the group consisting of a methyl group, an octyl group, a lauryl group, a cis-9-hexadecenyl group, a cis-9-octadecenyl group, a cis,cis-9,12-octadecadienyl group, a trans,trans-9,12-octadecadienyl group, a cis,cis,cis-9,12,15-octadecatrienyl group, a trans,trans,trans-9,12,15-octadecatrienyl group, a cis,cis,cis-6,9,12-octadecatrienyl group, a trans-9-octadecenyl group, and a trans-9-hexadecenyl group.

51. The method of claim 43, wherein said gallic acid ester is selected from the group consisting of (-)-epicatechin gallate, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, and tannic acid.

52. The method of claim 43, wherein the gallic acid ester is present in a range of 0.01 to 100 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

53. The method of claim 52, wherein the gallic acid ester is present in a range of 0.5 to 2 units of the gallic acid ester per 1 unit of the pharmaceutical compound.

54. The method of claim 43, wherein the reformulated oral composition comprises all components present in the existing pharmaceutical composition plus the gallic acid ester.

55. The method of claim 43, wherein the reformulated oral composition contains less than all components present in the existing pharmaceutical composition plus the gallic acid ester.

56. The method of claim 43, wherein the gallic acid ester comprises at least two of octyl gallate, lauryl gallate, and methyl gallate.

5 57. A reformulated oral pharmaceutical composition produced by the process of claim 43.

TOH200-1321700

**DECLARATION**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**USE OF GALLIC ACID ESTERS TO INCREASE BIOAVAILABILITY OF ORALLY ADMINISTERED PHARMACEUTICAL COMPOUNDS**

the specification of which:

(check one)

☐ is attached hereto;

☐ was filed as United States Application Serial No. \_\_\_\_\_ on \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable);

☒ was filed as PCT International Application No. PCT/US00/05524 on March 1, 2000 and was amended under PCT Article 19 or Article 34 on \_\_\_\_ (if applicable);

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above;

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information which is known to me to be material to the patentability of said invention in accordance with 37 C.F.R. §1.56;

I hereby claim foreign priority benefits under 35 U.S.C. §119 and/or §365 of any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America listed below; I have also identified below any foreign application(s) for patent, any foreign application(s) for inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Prior Foreign Application(s)

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			<input type="checkbox"/> YES <input type="checkbox"/> NO

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U.S. APPLICATIONS		STATUS (check one)		
U.S. APPLICATION NO.	U.S. FILING DATE (day, month, year)	Pending	Patented	Abandoned
09/264,215	March 5, 1999	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PCT APPLICATIONS DESIGNATING THE U.S.				
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



1-11  
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Vincent J. WACHER, et al.

Serial No.: Not yet assigned Examiner: Not yet assigned

Confirmation No.: Art Unit: Not yet assigned

Filed: Herewith

For: USE OF GALLIC ACID ESTERS TO INCREASE BIOAVAILABILITY OF ORALLY ADMINISTERED PHARMACEUTICAL COMPOUNDS

BOX PATENT APPLICATION

Commissioner for Patents  
Washington, D.C. 20231

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AND STATEMENT UNDER 37 C.F.R. §3.73(b)

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1

The Assignee certifies that to the best of its knowledge and belief it is the owner of the entire right, title, and interest in and to the above-identified application as evidenced by:

- [X] An assignment document, a copy of which is enclosed herewith;
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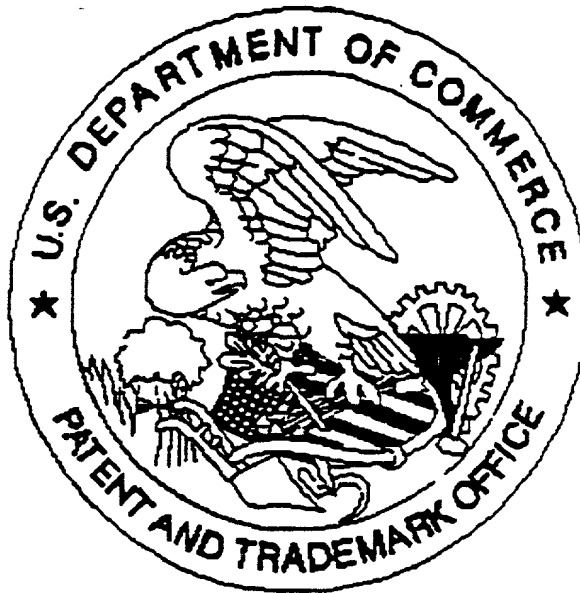
The undersigned (whose title is supplied below) is empowered to sign this statement on behalf of the Assignee.

Date: Aug 15, 2001

Signature: Leslie Z. Benet

Name: Leslie Z. Benet  
Title: President, CEO, and Chairman  
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South San Francisco, CA 94080

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